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TITLE: Cell-Based Meniscal Repair Using an Aligned Bioactive Nanofibrous Sheath

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of this proposal is to develop a novel bio-activated, aligned, nanofibrous scaffold that will serve as mechanical and biological support for the repair of radial tears of the meniscus. The hypothesis is that scaffolds consisting of aligned polymeric fibers, which structurally and mechanically mimic tendon and fibrocartilage, may be applied as a patch in alignment with the fibers of the tissue to be repaired, i.e., the meniscus with radial tear, to strengthen mechanically the surgical meniscal repair, and to subsequently guide tissue regeneration, for example, by seeded tissue progenitor cells.

To achieve this objective, the first step is to develop an aligned nanofibrous scaffold (NFS) that meets the mechanical requirements of the native meniscal matrix and provides suture retention. This will be done by combining nanofibers composed of FDA-approved biodegradable polymers to produce a biocompatible scaffold, which will provide mechanical support to the healing meniscus. To support suture retention, a second layer of non-aligned fibers will be coated onto the aligned fibers. Secondly, the NFS will be bio-enhanced by impregnation with an extract derived from decellularized meniscus matrix, which contains molecules and growth factors specific to this tissue, to increase the formation of fibrocartilage by adult stem cells seeded within the scaffold. This bio-activation should enhance the biological integration, i.e. adhesion, and tissue regenerating activity of the NFS in meniscus repair. Finally, we will test the ability of the bio-activated, aligned NFS sheath to enhance meniscus repair when combined with stem cell-based wound bonding strategies and standard suture repair using an in vitro model of meniscal repair (employed to elucidate the optimal combination of materials developed here) and in vivo, repairing surgically-induced radial defects in a goat.

15. SUBJECT TERMS

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TABLE OF CONTENTS

1. INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The meniscus is the most commonly injured structure of the knee, disproportionately affecting active populations such as military personnel. At present, removal of the torn tissues (i.e., partial meniscectomy) is the standard of care but predisposes the patient to rapid joint degeneration (i.e., osteoarthritis). Tissue engineering approaches, including the combination of cells, scaffolds, and bioactive agents (e.g., growth factors), have been explored as a means of bolstering the poor intrinsic healing capacity of the meniscus. Aligned electrospun nano/microfibers comprising engineered scaffolds can mimic the ultrastructure of the native meniscal extracellular matrix (ECM); when seeded with adult mesenchymal stem cells (MSCs), the nanofibers direct MSC orientation with corresponding upregulation of fibrochondrogenic differentiation. Similarly, photocrosslinkable hydrogels derived from natural ECM proteins (e.g., collagen, gelatin) can deliver MSCs under point-of-care procedures to a tear site and promote subsequent neotissue formation. Supplementation of these hydrogels with bioactive agents, such as growth factors or soluble ECM fractions, can enhance tissue-specific neotissue formation. The purpose of this project is to combine a biomimetic scaffold of electrospun nanofibers with a meniscal ECM-enhanced, MSC-seeded photocrosslinkable hydrogel to enhance healing of a radial meniscus tear, as evaluated in both an *in vitro* explant and *in vivo* goat model.

2. KEYWORDS: Provide a brief list of keywords (limit to 20 words).
Meniscus tissue engineering, electrospun scaffold, hydrogel, extracellular matrix, mesenchymal stem cell

3. ACCOMPLISHMENTS: The PI is reminded that the recipient organization is required to obtain prior written approval from the USAMRAA Grants Officer whenever there are significant changes in the project or its direction.

- What were the major goals and objectives of the project?
- What was accomplished under these goals?
- What opportunities for training and professional development did the project provide?
- How were the results disseminated to communities of interest?
- What do you plan to do during the next reporting period to accomplish the goals and objectives?

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Generally, the goals will not change from one reporting period to the next and are unlikely to change during the final reporting period. However, if the awarding agency approved changes to the goals during the reporting period, list the revised goals and objectives. Also explain any significant changes in approach or methods from the agency approved application or plan.

The project SOW contains three aims:

1. Develop aligned nanofibrous scaffold (NFS) to meet the mechanical requirements of the native meniscal matrix and provide suture retention
2. Develop and assess adult stem cell-seeded bioactivated NFS as a potential meniscal repair component
3. Verify the ability of the developed aligned NFS to promote meniscal repair in vitro and in a large animal model in vivo; the aligned NFS is to be combined with an ECM-enhanced photocrosslinkable hydrogel that will be injected into the tear site.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Accomplishments are organized within each aim:

1. **<u>Aim 1</u>: Develop aligned nanofibrous scaffold (NFS) to meet the mechanical requirements of the native meniscal matrix and provide suture retention**

Aim 1 Results:

Individual layers of poly-ε-caprolactone (PCL) nanofibers were electrospun in three orientations – aligned longitudinal, aligned transverse, and random (**Figure 1D-F**) – and combined as multilayered scaffolds of three patterns – aligned, random, and biomimetic (**Figure 1G-I**). The biomimetic scaffold contained, from deep to superficial, layers of aligned longitudinal, aligned transverse, aligned longitudinal, and random fibers, to mimic the circumferential, radial, and surface fiber ultrastructure of the native meniscus, respectively (**Figure 1**).

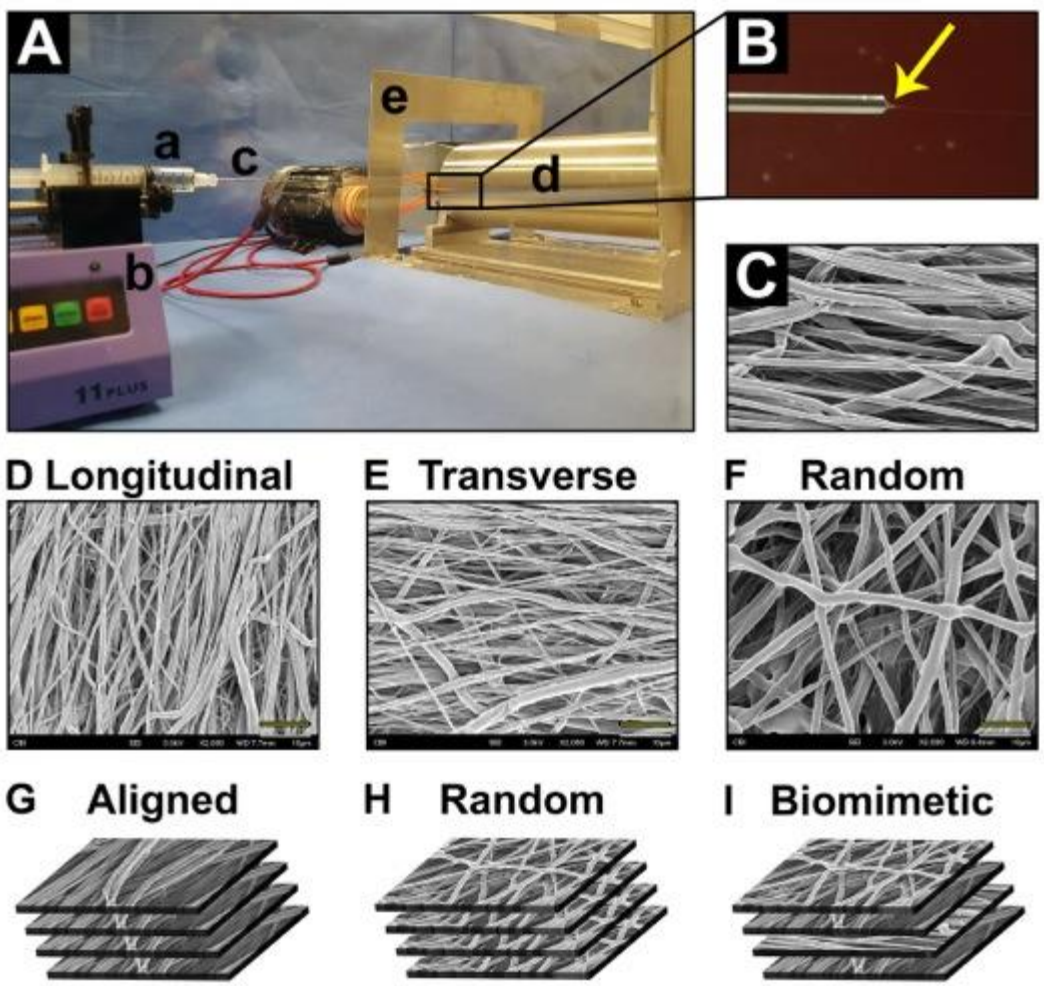


Figure 1. Fabrication of multilayered electrospun scaffolds. (A) Electrospinning apparatus consisting of (a) syringe with polymer solution, (b) syringe pump, (c) 18-gauge blunt tip needle, (d) rotating mandrel, and (e) aluminum shield. (B) Taylor cone (arrow) with emerging polymer fiber creates (C) nanofibrous sheet. (D-F) SEM images of fiber orientations comprising individual layers. Scale

bar, 10 μm. (G-I) Individual layers are combined to form three types of multilayered scaffolds, (G) aligned, (H) random, and (I) biomimetic (consisting of alternating layers of aligned and random layers).

The three multilayered scaffolds were loaded under uniaxial tension in both the parallel and perpendicular direction, and both structural and material properties were determined. The aligned scaffolds showed the greatest degree of anisotropy, with the highest modulus of all conditions when tension was applied in the direction of the PCL fibers (i.e., paralle). However, the biomimetic scaffold was not significantly weaker in the parallel direction than aligned scaffolds, but superior to other designs in the perpendicular direction (**Figure 2A**). Additionally, suture retention strength was highest in the biomimetic scaffold (**Figure 2B**)

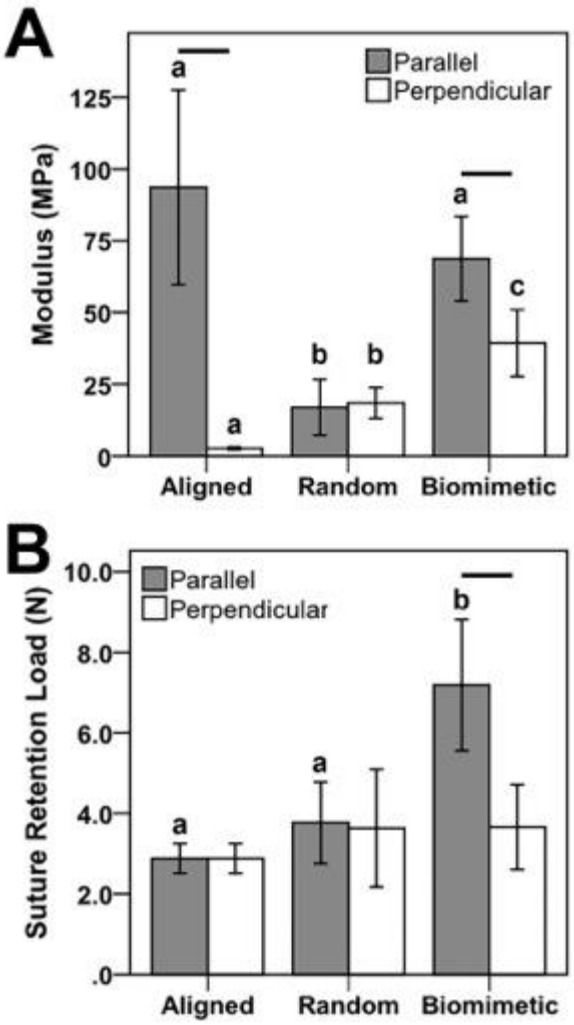
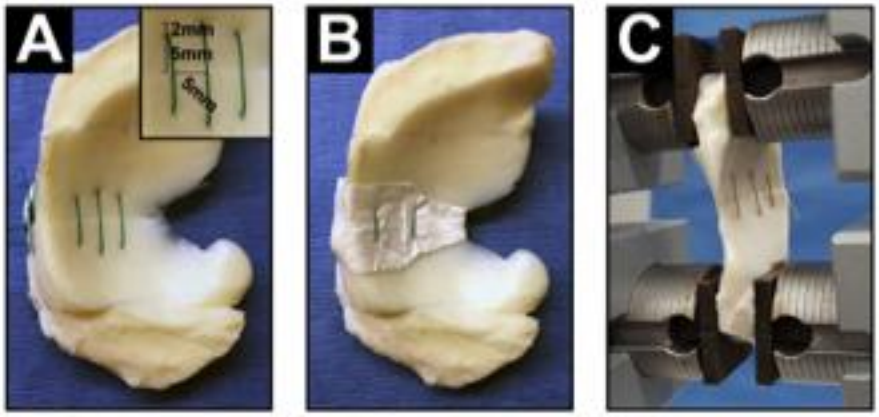


Figure 2. Moduli and suture retention strength of multilayered scaffolds. (A) Tensile modulus of three scaffold designs in parallel (i.e., circumferential) and perpendicular (i.e., radial) direction. (B) Ultimate suture retention load by scaffold design. * (p <0.05) and # (p <0.001) indicate significant difference across scaffold types for a given direction. Horizontal lines above columns indicate a significant difference (p < 0.001) between directions for a given scaffold type.

Given the superiority of the biomimetic scaffold design in terms of material properties and suture retention strength, it was incorporated as a sheath within a horizontal mattress suture repair of a radial tear simulated on a bovine meniscus explant (**Figure 3A,B**). The meniscus explanted was then cyclically loaded for 500 cycles (5N-20N) before loading to failure (**Figure 3C**). Testing revealed that the scaffold did not compromise, but did not improve, the residual gap formation of structural properties of the suture repair group (**Tables 1,2**).



Cycle	Native	Suture Repair	Scaffold-Augmented
1	0.26 ± 0.16[a]	1.14 ± 0.28	1.27 ± 0.38
10	0.40 ± 0.23[a]	1.75 ± 0.40	1.99 ± 0.33
50	0.55 ± 0.33[a]	2.57 ± 0.57	2.93 ± 0.35
100	0.66 ± 0.39[a]	3.15 ± 0.75	3.58 ± 0.47
250	0.86 ± 0.51[a]	4.29 ± 1.17	4.88 ± 0.80
500	0.93 ± 0.49[a]	4.78 ± 1.24	5.05 ± 0.89

	Native	Suture Repair	Scaffold-Augmented
Ultimate Load (N)[a]	437.3 ± 117.5	124.4 ± 21.4	137.1 ± 31.0
Ultimate Elongation (mm)[b]	5.12 ± 1.55	10.14 ± 4.61	12.09 ± 5.89
Stiffness (N/mm)	141.0 ± 42.4[a]	18.4 ± 4.7	20.8 ± 3.6

Figure 3. Suture repair of meniscal tears and mechanical testing set-up. (A) Suture repair of fully transected meniscus. Inset shows dimensions of suture placement. (B) Scaffold-augmented repair. (C) Suture repaired meniscus clamped in materials testing machine prior to tensile loading protocol. **Table 1. Residual Elongation (mm) During 500 Cycles Between 5N to 20N.** [a] Native control significantly less ($p < 0.001$) than either repair group at given cycle. **Table 2. Mechanical Properties of Native and Repaired Menisci Pulled to Failure.** [a] Native control significantly greater ($p < 0.001$) than either repair group. [b] Scaffold-augmented group significantly greater ($p = 0.022$) than native control.

Aim 1 Discussion:

We successfully developed an aligned electrospun nanofibrous scaffold (i.e., biomimetic) that approaches the material properties of native meniscus. The scaffold was stably incorporated as part of a clinically relevant suture repair, but did not enhance cyclic or load properties of the repair. The ultimate loads

and stiffness for repaired constructs (i.e., suture or scaffold-augmented groups) met or exceeded values reported in the literature. As all repairs failed by suture breakage, the limitation in enhancing the strength of suture repairs is the suture material itself, not the strength of the meniscus tissue nor the overlying scaffold. To that end, improving the material strength of the scaffold, or its suture retention strength, would NOT enhance the strength of the repair. Nevertheless, the inclusion of the scaffold could serve several purposes: (1) directing ECM deposition in the direction of electrospun fibers by endogenously recruited or exogenously seeded progenitor cells; (2) protecting an MSC-seeded hydrogel photocrosslinked within the tear site. For these reasons, we used the biomimetic scaffold design in subsequent aims.

2. **<u>Aim 2</u>: Develop and assess adult stem cell-seeded bioactivated NFS as a potential meniscal repair component**

Aim 2 Results:

Aim 2 assesses the ability of the NFS to support cell attachment and matrix deposition. As stated in the original project SOW, the NFS is further enhanced with a urea-extracted fraction of ECM derived from the ***outer*** meniscus. The NFS will be combined with an MSC-seeded photocrosslinkable hydrogel that could be further enhanced with a urea-extracted fraction of the ECM derived from the ***inner*** meniscus. To obtain the urea-extracted fraction of meniscus ECM, we adapted a protocol previously established in our lab for tendon ECM.[1] As shown in **Figure 4**, menisci from 6-8 week old bovine hindlimbs were halved, minced, crymilled, and solubilized through one of two methods – (1) urea extraction or (2) pepsin digestion. The urea-extracted fractions were enriched for small to medium weight non-collagenous proteins, while pepsin digestion produced a homogenous slurry that contained mostly collagen (**Figure 4E-H**).

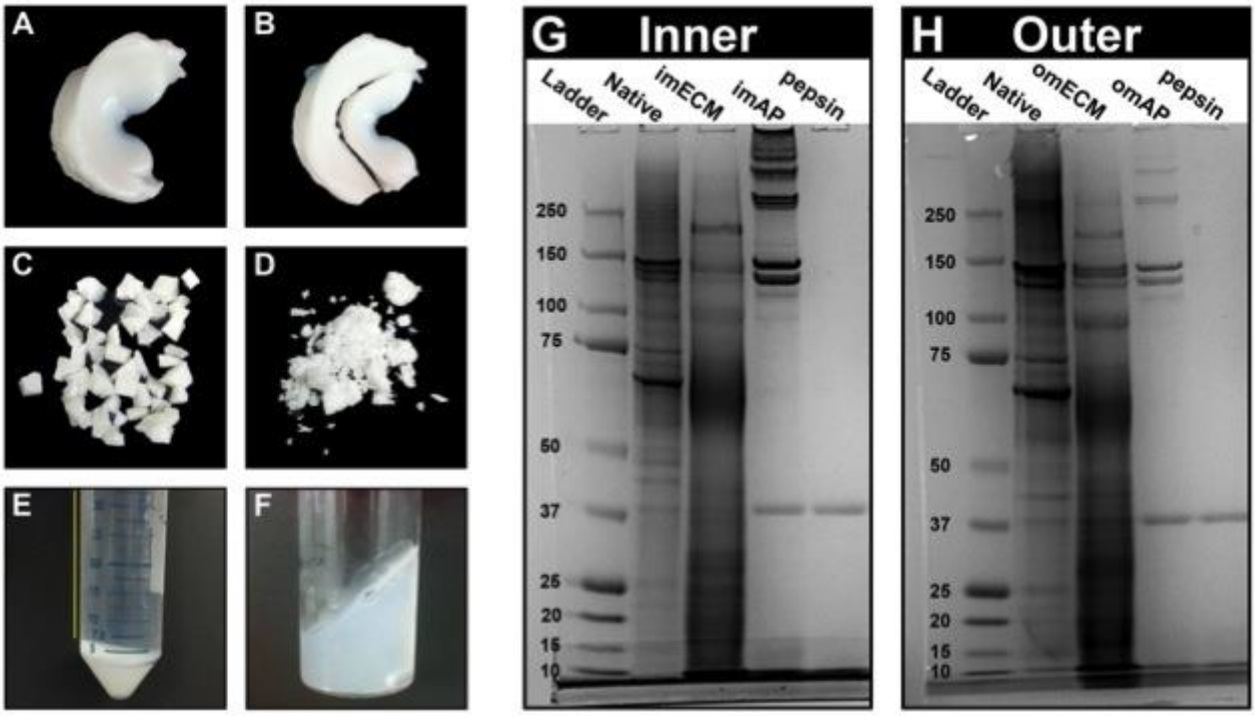


Figure 4. Solubilization of ECM from inner and outer meniscus. (A) Whole menisci were obtained from 6-8 week old cow hindlimbs, (B) halved, and (C) manually minced (8-27 mm^3). Following decellularization, (D) tissues were cryomilled and soluble fractions were obtained either by (E) urea-extraction (supernatant was retained, yellow line) or (F) acid-pepsin digestion. SDS-PAGE of (G) inner meniscus and (H) outer meniscus tissues and soluble preparations demonstrate that urea extraction retained low- and moderate-weight proteins while pepsin digestion yielded mostly collagen.

Preliminary studies revealed that pepsin-digested ECM did not promote region-specific differentiation of MSCs seeded on 2D plastic. Conversely, urea-extracted fractions of meniscus ECM supported increased cell proliferation (**Figure 5A-D**) and fibrochondrogenic differentiation. In particular, while both soluble ECMs derived from the inner meniscus (imECM) and outer meniscus (omECM) upregulated fibrochondrogenic

markers Sox9, Collagen type 2 (Col2), and Collagen type 1 (Col1), these results were more strongly promoted by imECM (**Figure 5E**).

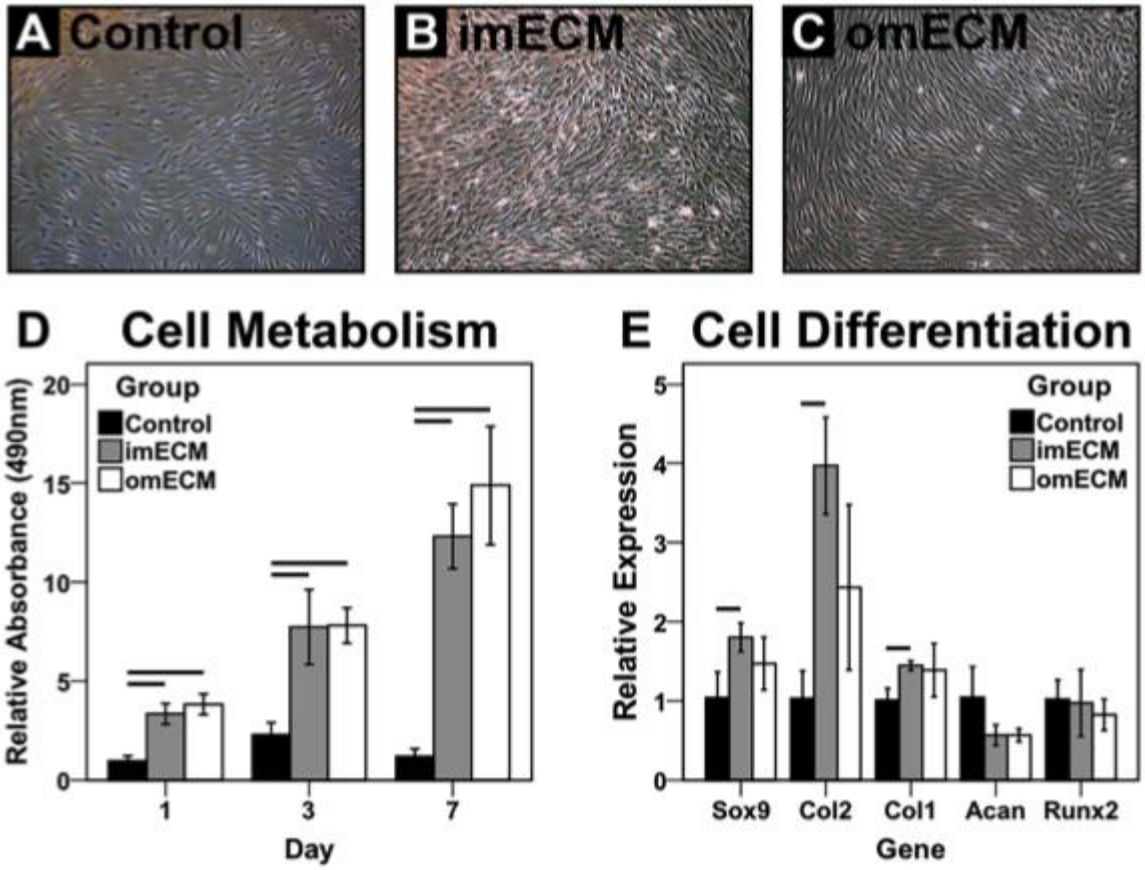


Figure 5. Bioactivity of soluble ECM extracts on MSCs in 2D culture. (A-C) phase contrast microscopy. (D) MTS assay measuring cell metabolism; n = 6-8 per condition; Lines indicate significant difference between groups on given day, $p < 0.05$. (E) Gene expression analysis on day 3; n=3 independent trials, each performed in biological triplicate; Lines indicate significant difference between groups, $p < 0.05$.

$15.0x10^6$ MSCs/mL were suspended in 10% w/v methacrylated gelatin (GelMA) hydrogels containing 0.25% photoinitiator (LAP) and supplemented with one of the following – (1) 1X PBS (Control), (2) 500 μg/mL imECM, or (3) 500 μg/mL omECM. Hydrogels were crosslinked by exposure to visible light (450-490nm) for 2 minutes and cultured for up to 42 days in chondrogenic medium. As shown in **Figure 6**, both imECM and omECM enhanced Col2 and proteoglycan deposition, with imECM being superior to omECM.

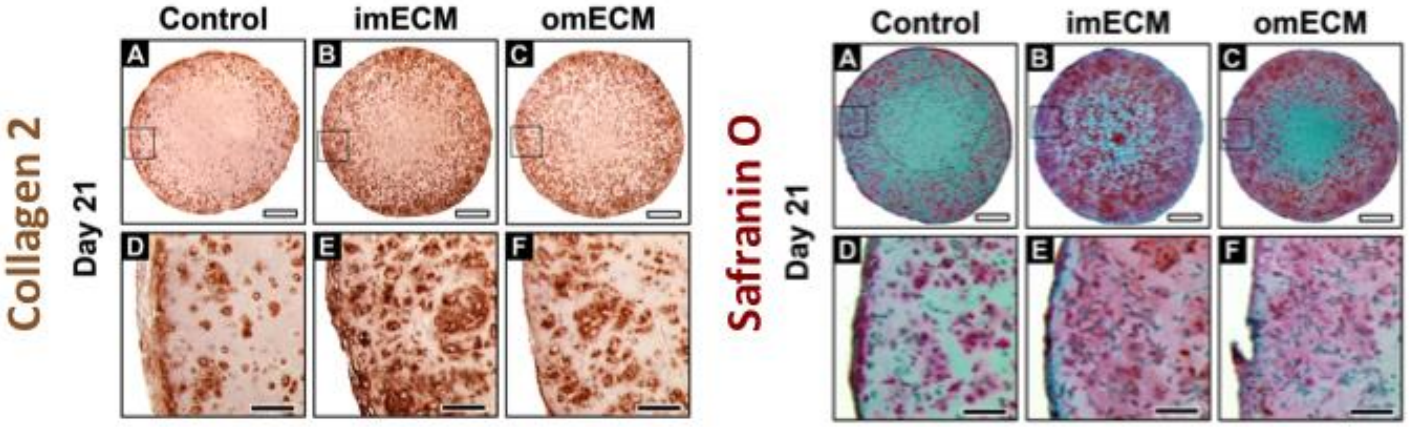


Figure 6. Immunohistochemical staining of Col2 (left) and histological staining (Safranin O/fast green; right) of ECM-enhanced GelMA hydrogels seeded with MSCs. Dark Brown = Col2; Red = Proteoglycan.

To explore the bioactivity of NFSs, MSCs were seeded on electrospun sheets of random or aligned nanofibers (**Figure 7**). The fiber orientation influenced cell morphology, with MSCs seeded on random fibers demonstrating no obvious directionality while those cultured on aligned fibers adopted an elongated morphology parallel to the fiber axis. Preliminary studies in which the PCL fibers were soaked in omECM showed relatively rapid loss of the omECM coating when exposed to culture medium, suggesting that protein adsorption is an insufficient method for stably functionalizing NFSs.

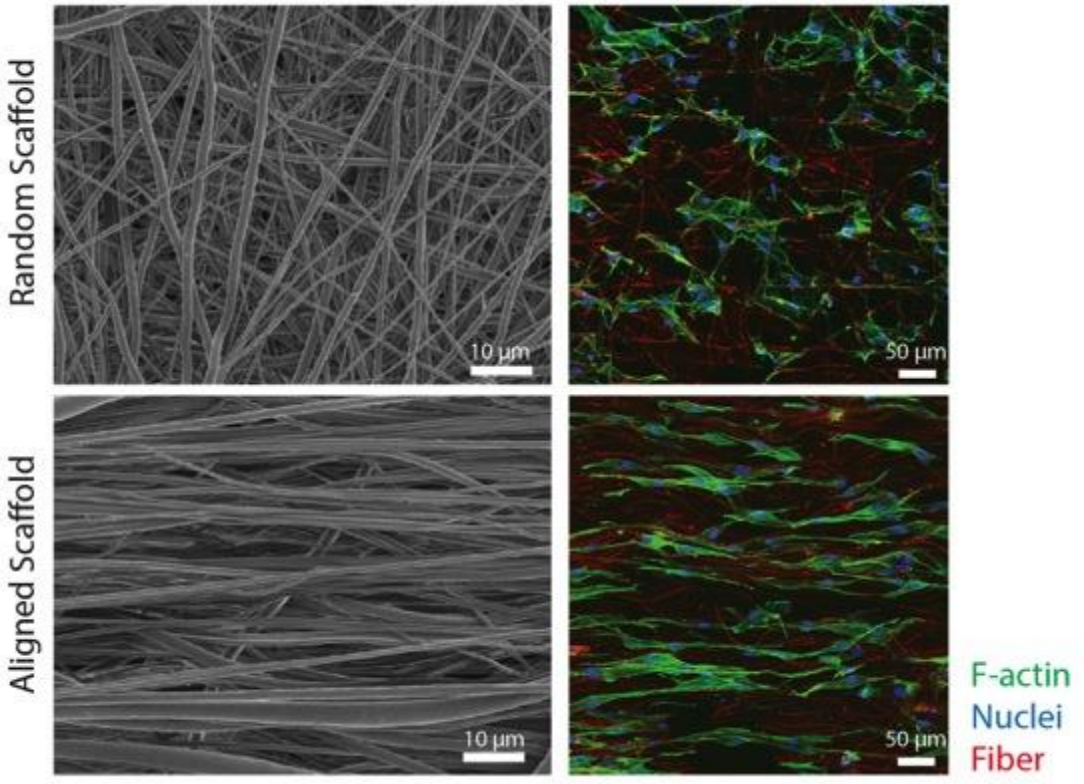


Figure 7. Morphology of MSCs cultured on scaffolds. MSCs seeded on random scaffold (upper left, SEM) exhibited polygonal shape without uniformity in orientation (upper right, confocal microscopy). In contrast, MSCs seeded on aligned scaffolds (lower left, SEM) adopted an elongated morphology and were orientated in the direction of the fibers (lower right, confocal microscopy; F-actin, green; nuclei, blue; microfiber, red).

Aim 2 Discussion:

The Biomimetic scaffold developed in Aim 1 was capable of supporting MSC attachment and orientation-mediated changes in cell morphology. While omECM and imECM enhanced fibrochondrogenic differentiation of MSCs cultured on 2D plastic or in photocrosslinkable GelMA hydrogels, this effect was stronger for imECM. Given the loss of omECM coating from NFSs when relying on protein adsorption, coupled with the weaker fibrochondrogenic effect of omECM (vs. imECM), we are pursuing Aim 3 (below) without further modification of the biomimetic scaffold. At the same time, imECM-enhanced GelMA hydrogels permit robust chondrogenic differentiation of encapsulated MSCs. Furthermore, encapsulating TGF-β within the hydrogel produces a comparable effect as adding TGF-β as a medium supplement, permitting us to use GelMA hydrogels containing imECM, TGF-β, and MSCs, in point-of-care repairs of meniscus tears.

3. **Aim 3: Verify the ability of the developed aligned NFS to promote meniscus repair *in vitro* and in a large animal model *in vivo*.**

Aim 3 Results:

Using an explant model of a radial meniscus tear, we've previously shown that MSC-seeded NFSs can enhance neotissue formation within the defect.[2] In employing the same model, we've now shown even better results when filling the defect with TGF-β-enhanced GelMA hydrogels (data not shown). Given these results, we are currently exploring the utility of NFS sheaths enveloping a radial tear filled with MSC-seeded TGF-β/imECM-enhanced hydrogels in a goat model (**Figure 8**). 14 goats will receive unilateral knee surgery, divided among the following groups – (1) Defect only (n=4), (2) Suture repair (n=5), (3) Augmented repair (n=5). Surgeries will be completed over the next 4 weeks.

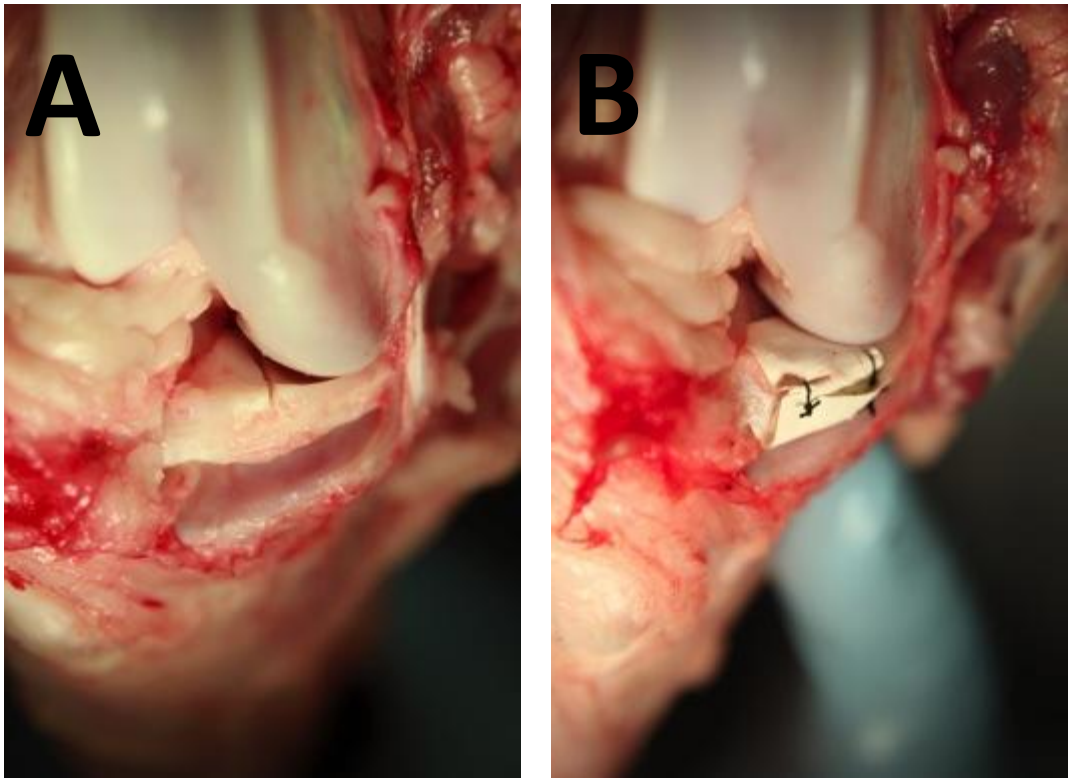


Figure 8. Goat model of radial meniscus tear. (A) A radial tear spanning 90% of the meniscus width is made at the junction of the anterior and middle body of the medial meniscus. (B) In the augmented repair group, the tear is filled with an MSC-encapsulated hydrogel, photocrosslinked, and wrapped with a biomimetic NFS.

Aim 3 Discussion:

In vitro explant models have shown tremendous promise of MSC-seeded GelMA hydrogels to promote healing in radial meniscus tears. The utility of these hydrogels, in combination with biomimetic NFSs, is being explored in a goat model, with the designated timepoint (6 months) occurring in January/February of 2017. As project funding ends December 2016, we are seeking a no-cost extension; the **request will be forthcoming.**

What opportunities for training and professional developproject provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Work from the project has been, or will be, presented at the following conferences:

1. Numpaisal P, Rothrauff BB, Lauro BB, Alexander PG, Debski RE, Musahl V, Tuan RS. "Augmented Repair of Radial Meniscus Tear with Biomimetic Electrospun Scaffold: An In Vitro Mechanical Analysis." 2016 Penn Cartilage Research Symposium, April 29-30, 2016. Philadelphia, PA
2. Rothrauff BB, Shimomura K, Gottardi R, Alexander PG, Tuan RS. "Encapsulation of Mesenchymal Stem Cells in Photocrosslinkable Hydrogel Enhanced with Meniscal Extracellular Matrix for Augmented Meniscus Repair." Military Health System Research Symposium, August 15-18, 2016. Kissimmee, FL.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Results of studies comprising the project have been, and will be, presented at national and international conferences, as outlined above.

One manuscript has been submitted, as follows:
Rothrauff BB, Numpaisal P, Lauro BB, Alexander PG, Debski RE, Musahl V, Tuan RS. Augmented Repair of Radial Meniscus Tear with Biomimetic Electrospun Scaffold: An In Vitro Mechanical Analysis. *Journal of Experimental Orthopaedics.*

Three additional manuscripts are in preparation and will be submitted within the next 6 months.

What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Within the next month, all 14 goats of Aim 3 will receive surgery. The goats will be sacrificed 6 months following the surgical date, at which time knees will be processed to access the quality of meniscus healing and resulting protection of articular surfaces. A request for no-cost extension is currently being drafted.

4. IMPACT: This component is used to describe ways in which the work, findings, and specific products of the project have had an impact during this reporting period. Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

- the development of the principal discipline(s) of the project;

- other disciplines;
- technology transfer; or
- society beyond science and technology.

Nothing to report

What was the impact on the development of the principal discipline(s) of the project?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to report

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (*Scientific American style*).

Nothing to report

How the field or discipline is defined is not as important as covering the impact the work has had on knowledge and technique. Make the best distinction possible, for example, by using a "field" or "discipline," if appropriate, that corresponds with a single academic department (i.e., physics rather than nuclear physics).

Nothing to report

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- transfer of results to entities in government or industry;
- instances where the research has led to the initiation of a start-up company; or
- adoption of new practices.

Nothing to report

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- improving public knowledge, attitudes, skills, and abilities;
- changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or
- improving social, economic, civic, or environmental conditions.

Nothing to report

5. CHANGES/PROBLEMS: The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

- Changes in approach and reasons for change.
- Actual or anticipated problems or delays and actions or plans to resolve them.
- Changes that have a significant impact on expenditures.
- Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

In aim 1, the mechanism of repair failure (i.e., suture breakage) suggested that further changes in scaffold design to enhance material properties and/or suture retention strength would not translate into improved surgical repair strength. Nevertheless, the biomimetic scaffold, when seeded with MSCs, was capable of supporting cell attachment, proliferation, and contact guidance of cell morphology. These findings, coupled with the promising results of MSC-seeded GelMA hydrogels enhanced with cECM and TGF-β in terms of promoting meniscus-specific neotissue formation, have caused us to pursue the following augmentation strategy *in vivo* – (1) an acellular biomimetic scaffold to envelop the tear site, in combination with (2) an autologous MSC-seeded TGF-β/cECM enhanced GelMA hydrogel localized to the tear site. This strategy, while slightly different from the originally approved protocol, does not change the objectives or scope of the project.

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Development and testing of the NFSs (Aim 1) and MSC-seeded hydrogel (Aim 2) took longer than 12 months, delaying the start of the *in vivo* experiments (Aim 3). With a 6 month endpoint, Aim 3 will extend beyond the 18-month funding period (December 2016), and additional time will be need to process the specimens and interpret results. The first animal surgeries have gone according to plan and we do not anticipate any additional delays or problems in completing the study. However, a no cost extension will be requested.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

The delay in beginning the *in vivo* experiments (Aim 3), as described above, will cause expenditures for animal housing and care to extend beyond the funding period. However, we anticipate being able complete the project with the approved budget total.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Two small modifications are now being submitted to the IACUC office, and will be subsequently sent to ACURO. These changes do not impact the execution or scope of the project and include:
(1) The use of goat coats post-operatively to reduce the risk of seroma formation at the abdominal site from which adipose tissue will be harvested
(2) Use of banamine and excede (in place of ketoprofen and cefazolin) for post-operative analgesia and anti-biosis. These changes were suggested by the house veterinarian and are currently employed under veterinarian exception.

6. PRODUCTS: List any products resulting from the project during the reporting period. Examples of products include:

- publications, conference papers, and presentations;
- website(s) or other Internet site(s);
- technologies or techniques;
- inventions, patent applications, and/or licenses; and
- other products.

If there is nothing to report under a particular item, state "Nothing to Report."

Nothing to report

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award. There is no restriction on the number. However, agencies are interested in only those publications that most reflect the work under this award in the following categories:

Nothing to report

Journal publications. List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Include any peer-reviewed publication in the periodically published proceedings of a scientific society, a conference, or the like. A publication in the proceedings of a one-time conference, not part of a series, should be reported under "Books or other non-periodical, one-time publications."

Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Books or other non-periodical, one-time publications. Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include

any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like.

Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Other publications, conference papers, and presentations. Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- data or databases;
- biospecimen collections;
- audio or video products;
- software;
- models;
- educational aids or curricula;
- instruments or equipment;
- research material (e.g., Germplasm; cell lines, DNA probes, animal models);
- clinical interventions;
- new business creation; and
- other.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Provide the following information on participants:

- what individuals have worked on the project?
- has there been a change in the other active support of the PD/PI(s) or senior/key personnel since the last reporting period?
- what other organizations have been involved as partners?

Nothing to report

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort).

- Provide the name and identify the role the person played in the project. Indicate the nearest whole person month (Calendar, Academic, Summer) that the individual worked on the project. Show the most senior role in which the person worked on the project for any significant length of time. For example, if an undergraduate student graduated, entered graduate school, and continued to work on the project, show that person as a graduate student, preferably explaining the change in involvement.

  Describe how this person contributed to the project and with what funding support. If information is unchanged from a previous submission, provide the name only and indicate "no change".

  Example:

Name:	Mary Smith
Project Role:	Graduate Student
Researcher Identifier (e.g., ORCID ID):	1234567
Nearest person month worked:	5
Contribution to Project:	Ms. Smith has performed work in the area of combined error-control and constrained coding
Funding Support:	The XYZ Foundation (Complete only if the funding support is provided from other than this award.)

Name:	Rocky S. Tuan
Project Role:	PI
Research Identifier:	University Employee ID# 124200
Nearest person month worked:	5% effort (0.60 Person Months)
Contribution to Project:	Dr. Tuan will have direct responsibility for the overall design and conduct of the study, oversight of data analysis and writing of publications and research reports. Dr. Tuan will supervise the day-to-day research activities of all personnel.
Funding Support:	N/A
Name:	Peter Alexander
Project Role:	Data Analyst Scientist
Research Identifier:	University Employee ID# 124097
Nearest person month worked:	7% effort (0.84 Person Months)
Contribution to Project:	Dr. Alexander's responsibilities will include biomaterial scaffold fabrication, cell isolation/propagation/characterization, tissue harvesting/repair/culture, and histological, biochemical, and mechanical analyses, and animal surgeries. He will work under close supervision of Dr. Tuan and will be involved in experimental design, data analysis, and the training of the postdoctoral fellow. He will also be involved in data analysis, and presentation of research findings in manuscripts and at scientific meetings.
Funding Support:	N/A
Name:	Alessandro Pirosa
Project Role:	Postdoctoral Associate
Research Identifier:	University Employee ID# 160892
Nearest person month worked:	40% effort (4.80 Person Months)
Contribution to Project:	Alessandro assists in the execution of the experiments in this project for all the proposed tasks, including biomaterial scaffold fabrication, cell isolation/propagation/characterization, tissue harvesting/repair/culture, and histological, biochemical, and mechanical analyses, and animal surgeries. He has been trained by Dr. Alexander, and will be supervised directly by Dr. Tuan and Dr. Alexander in all of his research activities, including experimental design, assays, and data analyses. He will also be responsible for safety requirement, material acquisition, protocol development, and handle reporting duties according to Department of Defense protocols.
Funding Support:	N/A
Name:	Benjamin Rothrauff
Project Role:	Graduate Student Researcher
Research Identifier:	University Employee ID# 130053
Nearest person month worked:	42% effort (5.040 Person Months)
Contribution to Project:	Ben assists in the execution of the experiments in this project for all the proposed tasks, including biomaterial scaffold fabrication, cell isolation/propagation/characterization, tissue harvesting/repair/culture, and histological, biochemical, and mechanical analyses, and animal surgeries. He has been trained by Dr. Alexander, and will be supervised directly by Dr. Tuan and Dr.

	Alexander in all of his research activities, including experimental design, assays, and data analyses. He will also be responsible for safety requirement, material acquisition, protocol development, and handle reporting duties according to Department of Defense protocols.
Funding Support:	N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Rocky Tuan:

The following previously active grant has closed:

Title: "Stem Cell-Based Neurotrophic Enhancement of an Aligned Nanofiber Scaffold for Nerve Repair"
Grant#: W81XWH-10-2-0084
Time Commitment: 0.60 calendar months (5% effort)
Role: PI
Supporting Agency: Department of Defense, Susan Dellinger, 820 Chandler St, Ft. Detrick MD, 21702
Performance Period: 9/1/2010 – 8/31/2014
Level of Funding: $418,638
Goals/Aims: This project aims to demonstrate that mesenchymal stem cells (MSCs) are able to

provide neurotrophic enhancement for nerve regeneration by stimulating axonal growth and Schwann cell migration across the injury gap.
Overlap: None

Title: "Development of Novel Point-of-Care Treatment for Articular Cartilage Injury"
Grant#: W81XWH-10-1-0850
Time Commitment: 0.60 calendar months (5% effort)
Role: Co-PI (PI: Dr. Hyun Joon Paek, Tissue Genesis Inc.)
Supporting Agency: Department of Defense, Susan Dellinger, 820 Chandler St, Ft. Detrick MD, 21702
Performance Period: 9/30/2010 – 9/29/2014
Level of Funding: $680,330
Goals/Aims: The overall objective of this project is to develop a point-of-care treatment option for articular cartilage injury, utilizing adipose stem cells and nanofibrous biomaterial scaffold, evaluated in both in vitro and in vivo animal models.
Overlap: None

Title: "AFIRM - Wake Forest / University of Pittsburgh Consortium"
Grant#: W81XWH-08-2-0032
Time Commitment: 2.60 calendar months (21.66% effort)
Role: Co-Principal Investigator and Co-Director (PI: Dr. Anthony Atala, Wake Forest University)
Supporting Agency: Department of Defense, Philip Huff, USAMRAA, 820 Chandler St., Fort Detrick, MD 21702
Performance Period: 3/10/2008 – 6/30/2014 (No cost extension requested)
Level of Funding: $18,300,000
Goals/Aims: The overall objective of this multi-institutional project is to accelerate regenerative solutions for the treatment of battlefield injuries. Administrative role.
Overlap: None

Title: "3-D Osteochondral Micro-tissue to Model Pathogenesis of Osteoarthritis"
Grant#: 5U18 TR000532-02
Time Commitment: 0.60 calendar months (5% effort)
Role: PI
Supporting Agency: National Institutes of Health, Ashley M. Norwood, 6001 Executive Boulevard, Bethesda, MD 20892
Performance Period: 7/1/2012 – 6/30/2015 (No cost extension)
Level of Funding: $721,450
Goals/Aims: For this project, we propose to construct an *in vitro* 3-dimensional microsystem that models the structure and biology of the osteochondral complex of the articular joint. Osteogenic and chondrogenic tissue components will be produced using adult human mesenchymal stem cells (MSCs) derived from bone marrow and adipose seeded within biomaterial scaffolds photostereolithographically fabricated with defined internal architecture.
Overlap: None

Title: "Regenerative Repair of Traumatic Articular Cartilage Injuries: Point-of-Care Application of Mesenchymal Stem Cells and Chondrocytes"
Grant#: W81XWH-08-2-0032
Time Commitment: 0.60 calendar months (5% effort)
Role: Project Leader (PI: Dr. Anthony Atala, Wake Forest University)
Supporting Agency: Department of Defense (AFIRM Seed project), Philip Huff, USAMRAA, 820 Chandler St., Fort Detrick, MD 21702
Performance Period: 11/1/2012 – 6/30/2015 (No cost extension requested)
Level of Funding: $294,276
Goals/Aims: This project will examine the potential efficacy of using a combination of adult stem cells and chondrocytes in the presence of platelet-rich plasma to repair articular cartilage defects.
Overlap: None

Title: "RiMED Fellows Program"

Grant#: No Identifier
Time Commitment: 0.47 calendar months (3.88% effort)
Role: Mentor/PI
Supporting Agency: RiMED Foundation, Sede legale: Via Bandiera 11 - 90133 Palermo, Italy. (Sharon Downey, U. of Pittsburgh contact for RiMED)
Performance Period: 6/29/2011 – 5/16/2015
Level of Funding: $53,046
Goals/Aims: The purpose of the RiMed Foundation is to act as the vessel for the Italian Government to train 50-60 Italian Post Doctoral Associates in University of Pittsburgh labs over the next several years so that they will comprise the vanguard generation of PIs in a new research institute in Italy, once it is constructed. Dr. Tuan serves as the Mentor for Dr. Riccardo Gottardi, an Italian Postdoctoral Associate. This project will continue to support the costs for Dr. Gottardi's fellowship at the Center for Cellular and Molecular Engineering.
Overlap: None

Title: "Development of Novel Bioartificial Ligament Using Autologous Biological Scaffold and Cells"
Grant#: W81XWH-13-2-0030
Time Commitment: 0.60 calendar (5% effort)
Role: Co-Investigator (PI: Dr. Hyun Joon Paek, Tissue Genesis Inc.)
Supporting Agency: Agency: Department of Defense, Grants Officer – TBN, USAMRAA, 820 Chandler St., Fort Detrick, MD 21702
Performance Period: 8/1/13 – 4/28/15
Level of Funding: $400,000
Goals/Aims: The ultimate goal of this project is to develop novel cell-based therapies to treat injured ligaments and tendons that affect millions of Americans each year, using autologous biological materials.
Overlap: None

<u>Previously pending grant that are now active:</u>

Title: "Customized Fabrication of Osteochondral Tissue for Articular Joint Surface Repair"
Grant#: OR130296
Time Commitment: 1.20 calendar (10% effort)
Role: PI
Supporting Agency: Department of Defense, Grants Officer – TBN, USAMRAA, 820 Chandler St., Fort Detrick, MD 21702
Performance Period: 9/1/14 – 8/31/16
Level of Funding: $770,000
Goals/Aims: This study aims to test the hypothesize that cell-laden scaffolds loaded with microparticles designed to provide temporospatially specific differentiation cues for chondrogenesis and osteogenesis may be constructed by the 3D printing method of projection stereolithography (PSL) to produce functional osteochondral constructs using adipose stem cells.
Overlap: None

Title: "Treatment of Orthopaedic Infections using Activated Adult Mesenchymal Stem Cells"
Grant#: DM140372
Time Commitment: 1.20 calendar (10% effort)
Role: PI
Supporting Agency: Department of Defense, Grants Officer – TBN, USAMRAA, 820 Chandler St., Fort Detrick, MD 21702
Performance Period: 9/1/14 – 8/31/17
Level of Funding: $1,141,508
Goals/Aims: In this proposal we will verify that lipopolysaccharide-activated adipose tissue-derived stromal/stem cells possess anti-microbial activity through the production of LL-37. We will then develop a device that will expose adipose tissue-derived stromal/stem cells to lipopolysaccharide in a controlled manner over a short period (hours or minutes) to activate the antimicrobial functions of adipose tissue-derived stromal/stem cells.
Overlap: None

Title: "Vanderbilt-Pittsburgh Resource for Organotypic Models for Predictive Toxicology (VPROMPT)"
Grant#: Identifier Pending
Time Commitment: 0.90 calendar (7.5% effort)
Role: Project Principal Investigator (PI: Dr. Shane Hutson, Vanderbilt University)
Supporting Agency: Environmental Protection Agency, Grants Officer – TBN, 1200 Pennsylvania Ave NW, Washington, DC 20004
Performance Period: 11/1/14 – 10/31/18
Level of Funding: $1,000,000
Goals/Aims: Our objective is to develop two robust in vitro three-dimensional (3D) organotypic microculture models (OCMs) based on human mesenchymal stem cells (hMSCs) to examine three critical phenomena of embryonic limb development that are prime targets of limb teratogenesis, and their susceptibility to perturbation by candidate toxicants/teratogens.
Overlap: None

Title: "Adult Stem Cell-Based Neurotrophic Conduit Enhancement of Peripheral Nerve Repair"
Grant#: JW140121
Time Commitment: 1.80 calendar (15% effort)
Role: PI
Supporting Agency: Department of Defense, Grants Officer – TBN, USAMRAA, 820 Chandler St., Fort Detrick, MD 21702
Performance Period: 12/1/14 – 11/30/18
Level of Funding: $2,407,903
Goals/Aims: This proposal aims to apply adult stem cells (MPCs) as a neurotrophic agent in the development of a bioactivated nerve conduit for the repair of injured peripheral nerves, using both a small animal sciatic nerve repair model and a clinically relevant large animal model. The final goal is to prepare a combination product IDE application to the Food and Drug Administration for a clinical trial in wounded warriors or civilian patients with critical nerve gaps.
Overlap: None

Pete Alexander:

The following previously active grant has closed:

Title: "Stem Cell-Based Neurotrophic Enhancement of an Aligned Nanofiber Scaffold for Nerve Repair"
Grant#: W81XWH-10-2-0084
Time Commitment: 0.96 calendar months (8% effort)
Role: Co-Investigator (PI: Dr. Rocky Tuan)
Supporting Agency: Department of Defense, Susan Dellinger, 820 Chandler St, Ft. Detrick MD, 21702
Performance Period: 9/1/2010 – 8/31/2014
Level of Funding: $418,638
Goals/Aims: This project aims to demonstrate that mesenchymal stem cells (MSCs) are able to provide neurotrophic enhancement for nerve regeneration by stimulating axonal growth and Schwann cell migration across the injury gap.
Overlap: None

Title: "Development of Novel Point-of-Care Treatment for Articular Cartilage Injury"
Grant#: W81XWH-10-1-0850
Time Commitment: 4.80 calendar months (40% effort)
Role: Data Analyst Scientist (PI: Dr. Hyun Joon Paek, Tissue Genesis Inc.)
Supporting Agency: Department of Defense, Susan Dellinger, 820 Chandler St, Ft. Detrick MD, 21702
Performance Period: 9/30/2010 – 9/29/2014

Level of Funding: $680,330
Goals/Aims: The overall objective of this project is to develop a point-of-care treatment option for articular cartilage injury, and the project will be divided into three phases. Phase I: Evaluation of adipose stem cells (ASCs) and biomaterial scaffold; Phase II: Evaluate combination product in animal models; Phase III: IRB Study preparation.
Overlap: While the goals are somewhat similar, there is no scientific overlap since this funded project proposes to evaluate pre-formed nanofibrous scaffold, not 3D photocrosslinked hydrogel nor the use of microparticles for growth factor delivery, for the repair of only the articular cartilage tissue. The knowledge gained from this funded project, which ends in 6 months, will help to inform our proposed approach in the project that is recommended for funding.

Title: "3-D Osteochondral Micro-tissue to Model Pathogenesis of Osteoarthritis"
Grant#: 5U18 TR000532-02
Time Commitment: 0.72 calendar months (6% effort)
Role: Data Analyst Scientist (PI: Dr. Rocky S. Tuan)
Supporting Agency: National Institutes of Health, Tijuana Decoster, 6001 Executive Boulevard, Bethesda, MD 20892
Performance Period: 7/1/2012 – 6/30/2015 (No cost extenstion)
Level of Funding: $721,450
Goals/Aims: For this project, we propose to construct an *in vitro* 3-dimensional microsystem that models the structure and biology of the osteochondral complex of the articular joint. Osteogenic and chondrogenic tissue components will be produced using adult human mesenchymal stem cells (MSCs) derived from bone marrow and adipose seeded within biomaterial scaffolds photostereolithographically fabricated with defined internal architecture.
Overlap: None

Title: "Regenerative Repair of Traumatic Articular Cartilage Injuries: Point-of-Care Application of Mesenchymal Stem Cells and Chondrocytes" – Project in AFIRM I
Grant#: W81XWH-08-2-0032
Time Commitment: 0.90 calendar months (7.5% effort)
Role: Data Analyst Scientist (Project Leader: Dr. Rocky Tuan; PI: Dr. Anthony Atala, Wake Forest University)
Supporting Agency: Department of Defense (AFIRM Seed project), Philip Huff, USAMRAA, 820 Chandler St., Fort Detrick, MD 21702
Performance Period: 11/1/2012 – 6/30/2015 (No cost extension)
Level of Funding: $294,276
Goals/Aims: This project will examine the potential efficacy of using a combination of adult stem cells and chondrocytes in the presence of platelet-rich plasma to repair articular cartilage defects.
Overlap: This project will end in 3 months, with primary focus on the biological activity of platelet-rich plasma and cartilage fragments. No scientific overlap.

<u>Previously pending grant that are now active:</u>

Title: "Customized Fabrication of Osteochondral Tissue for Articular Joint Surface Repair"
Grant#: OR130296
Time Commitment: 4.80 calendar (40% effort)
Role: Data Analyst Scientist (PI: Dr. Rocky Tuan)
Supporting Agency: Department of Defense, Grants Officer – TBN, USAMRAA, 820 Chandler St., Fort Detrick, MD 21702
Performance Period: 9/1/14 – 8/31/16
Level of Funding: $770,000
Goals/Aims: This study aims to test the hypothesize that cell-laden scaffolds loaded with microparticles designed to provide temporospatially specific differentiation cues for chondrogenesis and osteogenesis may be constructed by the 3D printing method of projection stereolithography (PSL) to produce functional osteochondral constructs using adipose stem cells.
Overlap: None
Title: "Vanderbilt-Pittsburgh Resource for Organotypic Models for Predictive Toxicology (VPROMPT)"
Grant#: Identifier Pending
Time Commitment: 2.40 calendar (20% effort)

Role: Data Analyst Scientist (Project PI: Dr. Rocky Tuan; Principal Investigator: Dr. Shane Hutson, Vanderbilt University)
Supporting Agency: Environmental Protection Agency, Grants Officer – TBN, 1200 Pennsylvania Ave NW, Washington, DC 20004
Performance Period: 11/1/14 – 10/31/18
Level of Funding: $1,000,000
Goals/Aims: Our objective is to develop two robust in vitro three-dimensional (3D) organotypic microculture models (OCMs) based on human mesenchymal stem cells (hMSCs) to examine three critical phenomena of embryonic limb development that are prime targets of limb teratogenesis, and their susceptibility to perturbation by candidate toxicants/teratogens.
Overlap: None

Title: "Adult Stem Cell-Based Neurotrophic Conduit Enhancement of Peripheral Nerve Repair"
Grant#: JW140121
Time Commitment: 1.20 calendar (10% effort)
Role: Co-Investigator
Supporting Agency: Department of Defense, Grants Officer – TBN, USAMRAA, 820 Chandler St., Fort Detrick, MD 21702
Performance Period: 12/1/14 – 11/30/18
Level of Funding: $2,407,903
Goals/Aims: This proposal aims to apply adult stem cells (MPCs) as a neurotrophic agent in the development of a bioactivated nerve conduit for the repair of injured peripheral nerves, using both a small animal sciatic nerve repair model and a clinically relevant large animal model. The final goal is to prepare a combination product IDE application to the Food and Drug Administration for a clinical trial in wounded warriors or civilian patients with critical nerve gaps.
Overlap: None

If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission.

Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state "Nothing to Report."
Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner's contribution to the project (identify one or more)

- Financial support;

- In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);
- Facilities (e.g., project staff use the partner's facilities for project activities);
- Collaboration (e.g., partner's staff work with project staff on the project);
- Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and
- Other.

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS: None

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

REFERENCES

1. Yang G, Rothrauff BB, Lin H, et al. 2013. Enhancement of tenogenic differentiation of human adipose stem cells by tendon-derived extracellular matrix. Biomaterials 34: 9295-9306.
2. Shimomura K, Bean AC, Lin H, et al. 2015. In Vitro Repair of Meniscal Radial Tear Using Aligned Electrospun Nanofibrous Scaffold. Tissue Eng Part A 21: 2066-2075.